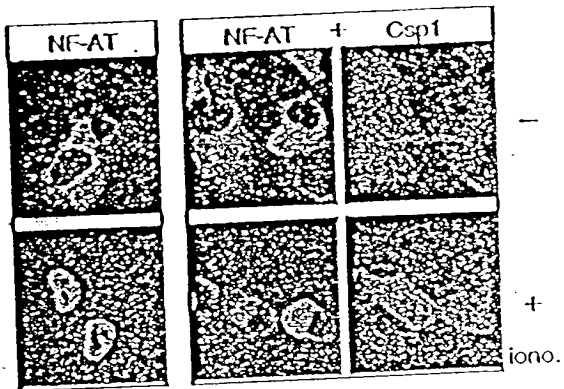
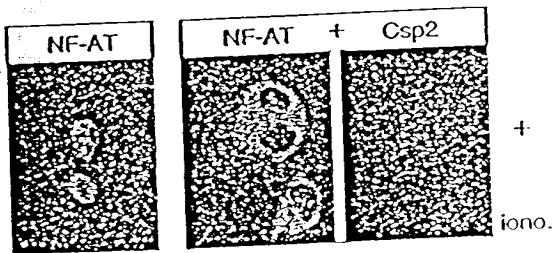




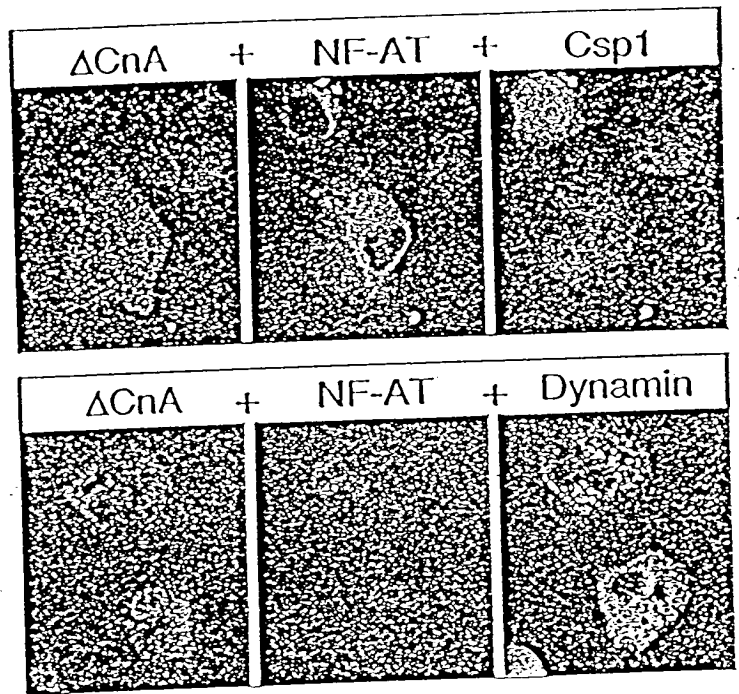
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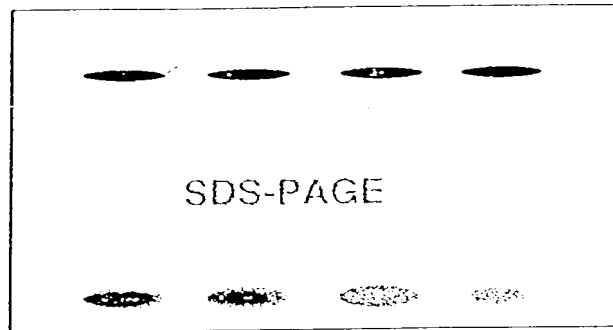
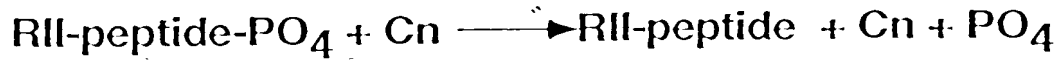
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C.

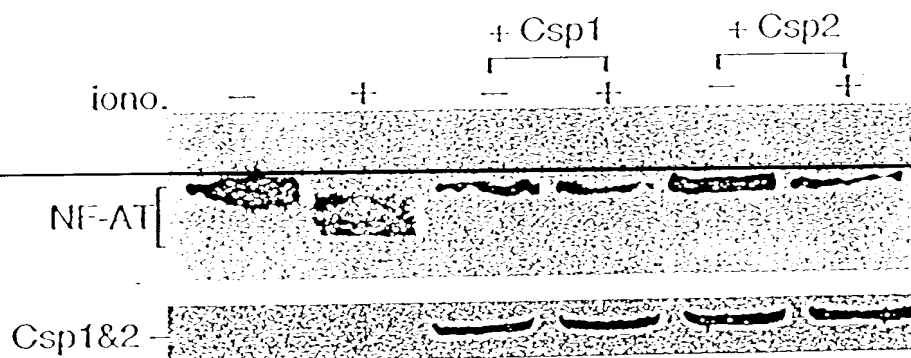


A.

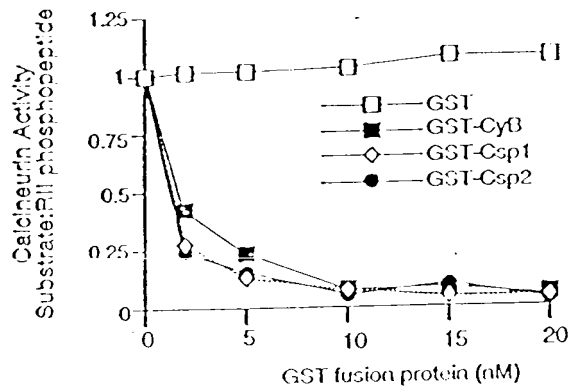


—free PO<sub>4</sub>  
(Quantitated with  
Phosphur-imager)

B.



A.



B.

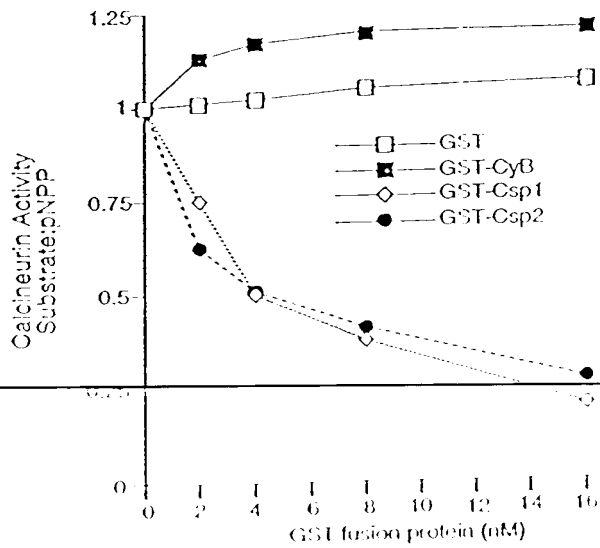
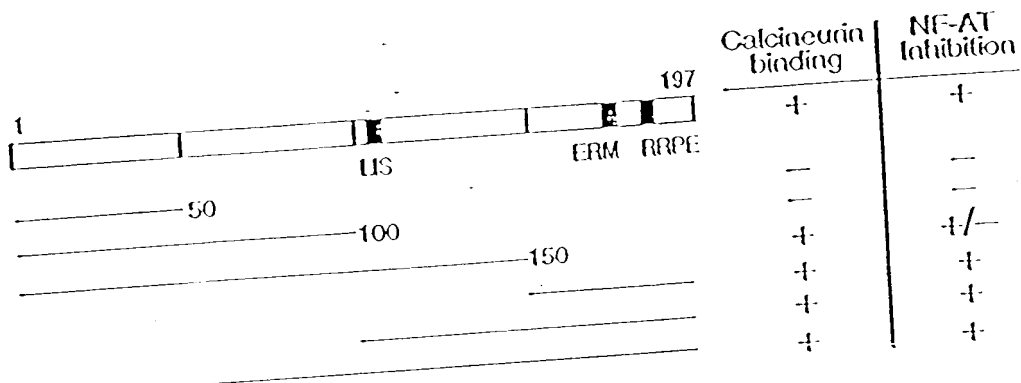
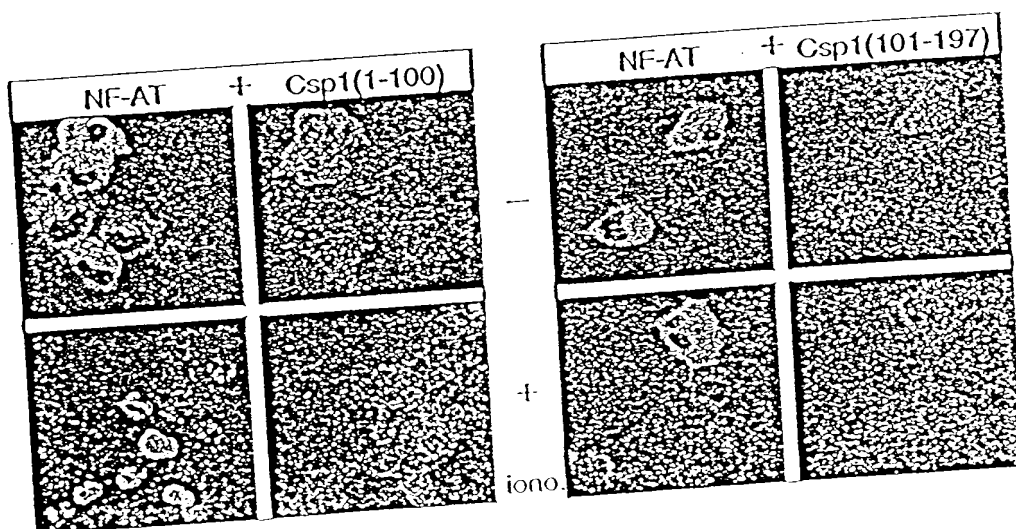


FIGURE 3

A.



B.



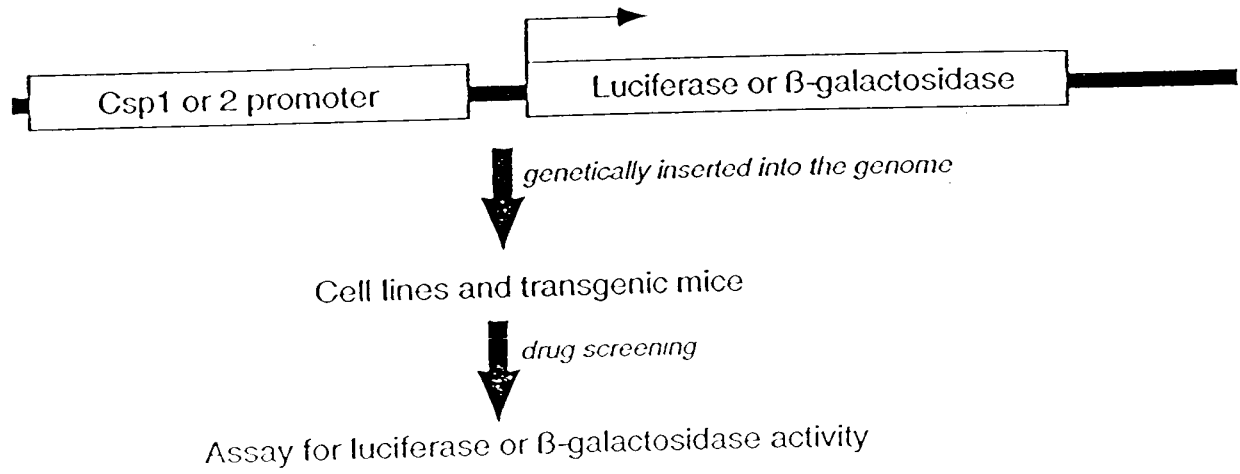
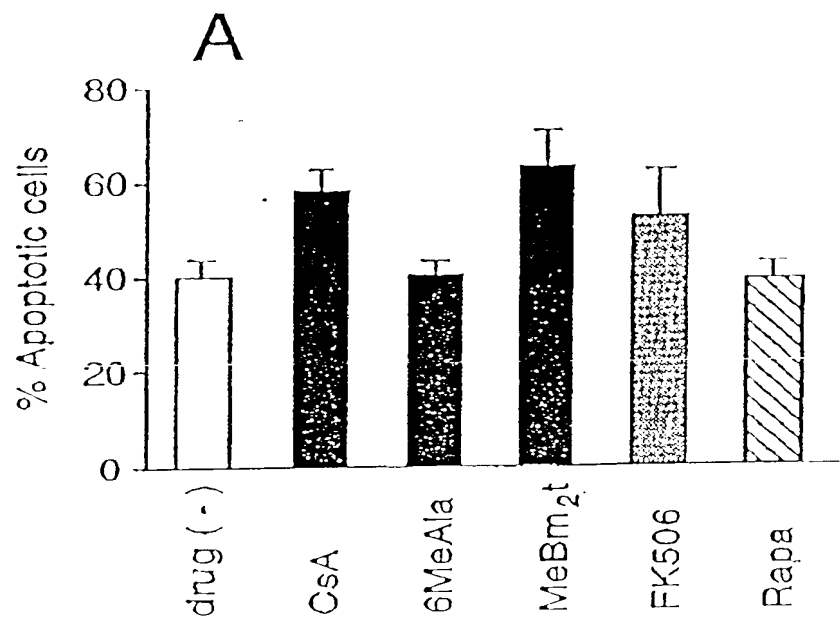
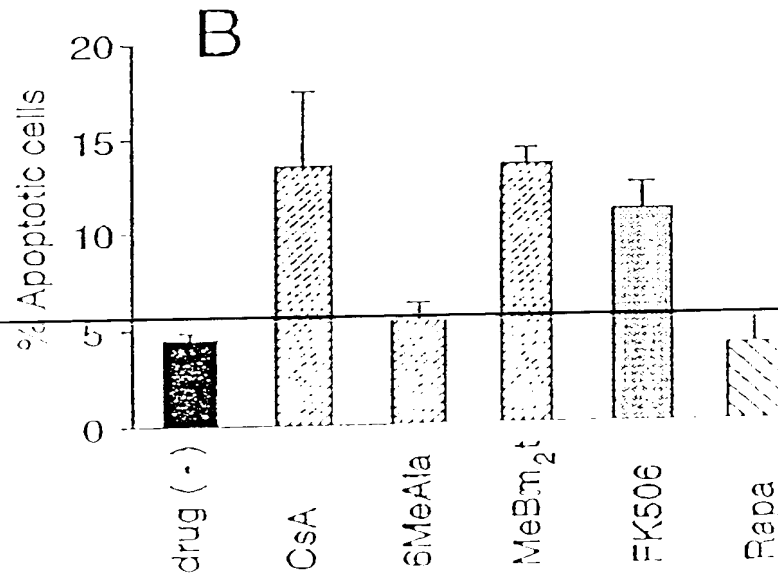
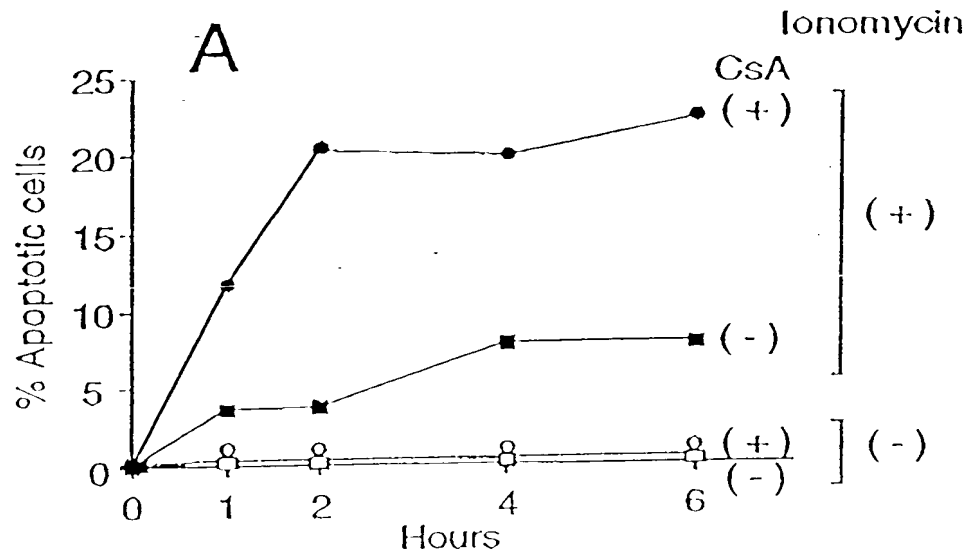


Figure 6







## human Csp1 promoter (2.5kb) (SEQ ID NO: 1)

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101	ggatataactt	tttgttataa	ttactaacac	ttcctaacta	gagagtaagc	200	
	clactctaag	aaaaaatata	actgtaattt	cacaacctcc	aaagaaccca		
201	gtgcataaac	agctaccatt	tattaagcac	tgactgaatt	cttagtaata	300	MyoD, NF-AT
	tgtcttcatt	<u>tttttcagat</u>	<u>gaggaaacta</u>	agattcagct	tatttgtaca		
301	agtagttaaa	aagcaaagct	gaaattcaga	cccaagttct	caactgtatca	400	
	tactgtccaa	aaaagaattc	tatttttcag	gaagagacat	gtctgtcac		
401	ttgaggctct	<u>cttatttttc</u>	cgctattccc	<u>caagggaag</u>	gggtgatctc	500	NF-AT, NF-AT
	ttaattcttt	cgttatgtcc	tattgtacat	agcatataat	ggtaattcag		
501	aaaaattact	tctaattaca	taaattttca	caatgggtata	gtgactaata	600	NF-AT
	<u>cgctgaata</u>	gaaaaqtaag	gcattgttat	catgggtctag	ttcagctctt		
601	attgcgacta	tatctgataa	tatacggtaa	gcactaacc	acttgccagg	700	
	ggccacagag	ccacagggag	actatgtctc	gcttaaattc	ccaaaagtgg		
701	gccccgtgtc	ttcaaaacgt	ccccgcattg	gaaccacaaa	aacgttgctt	800	
	ccccagttat	caccccaagg	gcccaagagc	cgaggactct	gcccggcgtc		MyoD
801	cttcagctgg	<u>caccagctgt</u>	cagaaaagcg	gaactgggga	cgaggacttt	900	
	gccccaaacc	<u>aacatggccg</u>	ccctgaggct	tcgggcttcg	ggcggcagaa		MyoD
901	ggaaggtcac	<u>gtgaagagaa</u>	ttccgttctc	ttattggccc	cgtctcctgg	1000	
	aagggcgggg	tacaataacc	caaccggcgc	cgcccttaa	ggggccaccg		
1001	ttggatctgc	cggtggcccg	ccctaggggc	tgggggggcg	gtcgccgcgc	1100	
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1101	ggaggccgtg	tcgctgggag	actgctgaca	gcccccgcc	tgccgcccgc	1200	
	cgattccgag	gggggttaacg	gcggaagccg	cgcccgggcg	cggaccggag		
1201	cgcgtgaggc	tccggcgccg	aagcccgag	cagcccgctg	ggcgccacag	1300	
	ggtegcgcgg	gcgcggggat	ggaggacggc	gtggccggtc	cccagctcgg		
1301	ggccgcggcg	gaggcgccgg	aggcgccga	ggcgcgagcg	cggcccgggg	1400	
	tgaecgtcgc	gcccttcgcg	ccccctcgg	ggcgcccgca	ggcgacgag		
1401	ggcgcgccgg	actggagctt	cattgactgc	gagatggagg	aggtggacct	1500	MyoD
	gcaggacctg	cccagcgcca	ccatcgccctg	<u>tcacctggag</u>	cccgccgtgt		
1501	tcgtggacgg	ctgtgtccgg	gtgaggaccg	cgcggggcgg	gccgtcgggg	1600	MyoD
	cggaggcgcg	<u>acacttgttg</u>	cccaggaggg	cggcgccggg	cgcagcgccc		
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	cggcccgagg	cccgtcaggg	ctggggcggt	gggacggcgc	cccaggggtc		
1701	ccggteccct	agcacccecg	ggcgcgccgg	agctcactgc	agagteccac	1800	
	aggetcgcgc	cggcccccg	gtgcgcgccg	gctgggtgcga	ctaggggggt		
1801	gaattcgctc	cccaagggtg	ggcagcgccg	ccgccecttg	cgtctctgcg	1900	
	<u>atcgccccgc</u>	<u>atttactcgc</u>	<u>tggaggaggg</u>	<u>ggtcacctca</u>	<u>ttcctagggg</u>		
1901	ggaggaaaaca	gacattgagc	gcccacgtga	ctcagtttcc	atanaatagga	2000	
	cgaagteccct	gcattcccac	tctgcactat	tggagaataa	qccaatqttt		
2001	qqqtgaqnat	ccgtqqttgc	tcattagcca	gcqctqgccc	agtttttqgtg	2100	
	qaatttgtgtt	gggggggaagg	ggaacatctt	tcagaccttt	agpatattta		
2101	gtcaagaacc	ttgccccctt	gtgtgaaggt	qtqcttgcgc	qccatcgggg	2200	
	acaccacgta	catggggaggt	cgactccttc	ccccgcctcc	ccccaccccc		
2201	qcaaaaatcca	cacaatttag	acacttttga	gggtgagggg	cagggtatgag	2300	
	taatcaataa	tgtgtgtggg	gaggaagaat	ttatttcaaa	tctgcagtta		
2301	ttgtgcagaa	taaaatgtgg	acaacgtggg	cgtcacagaa	tgaacccggg	2400	
	cttttgagaga	tgccecatla	ggagagcagc	tgtcaaaaaa	agcagtgctt		
2401	tcaagccttg	qctgtqqgtc	cacaaatgct	gtcaatqaac	tatagttga	2484	
	qqctgctqcc	aatacaaacac	cactgtqaaa	caga			

murine Csp1 (SFQ ID NO: 2)

```

1                               31
ATG GAG GAG GTG GAT CTG CAG GAC CTG CCG AGC GCC ACC ATC GCC TGC CAC CTG GAC CCG
61                               91
CGC GTG TTC GTG GAC GGC CTG TGC CGG GCC AAA TTT GAA TCC CTC TTC AGA ACA TAT GAC
121                              151
AAG GAC ACC ACC TTC CAG TAT TTT AAG AGC TTC AAA CGT GTC CGG ATA AAC TTC AGC AAC
181                              211
CCC TTA TCT GCA GCC GAT GCC AGG CTG CGG CTG CAC AAG ACC GAG TTC CTG GGG AAG GAA
241                              271
ATG AAG TTG TAT TTT GCT CAG ACT TTA CAC ATA GGA AGT TCA CAC CTG GCT CCG CCC AAT
301                              331
CCC GAC AAA CAG TTC CTC ATC TCC CCT CCG GCC TCT CCT CCC GTT GGC TGG AAA CAA GTA
361                              391
GAA GAT GCC ACC CCC GTC ATA AAT TAC GAT CTT TTA TAT GCC ATC TCC AAG CTG GGG CCA
421                              451
GGA GAG AAG TAT GAA CTG CAT GCA GCG ACA GAC ACC ACT CCC AGT GTG GTG GTC CAC GTG
481                              511
TGT GAG AGT GAC CAA GAG AAT GAG GAG GAA GAG GAA GAG ATG GAG AGA ATG AAG AGA CCC
541                              571
AAG CCC AAA ATC ATC CAG ACA CGG AGA CCG GAG TAC ACA CCC ATC CAC CTC AGC TGA

```

coding sequence: 597 nucleotides

murine Csp2 (SEQ ID NO: 3)

```

1      31
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61      91
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121     151
TTT GCT GAA GAG GCC TTC CAA GCA CTC ACT GAC TTC AGT GAT CTC CCC AAC TCA TTG TTT
181     211
GCC TGC AAT GTT CAC CAG TCT GTG TTT GAA GAA GAG GAG AGC AAG GAA AAA TTC GAG GGA
241     271
CTG TTC CGG ACC TAT GAT GAA TGT GTG ACG TTC CAG CTG TTT AAG AGT TTC CGA CGG GTT
301     331
CGA ATA AAT TTC AGC CAT CCC AAA TCT GCA GCC CGT GCC CGG ATA GAG CTT CAT GAG ACT
361     391
CAG TTC AGA GGG AAG AAG CTA AAA CTC TAC TTC GCC CAG GTC CAG ACC CCA GAG ACA GAT
421     451
GGA GAC AAA CTG CAT TTG GCA CCT CCA CAG CCT GCC AAA CAG TTC CTC ATC TCA CCC CCT
481     511
TCA TCT CCA TCT GTT GGC TGG AAG CCT ATC AGC GAT GCC ACA CCA GTC CTC AAC TAT GAC
541     571
CTT CTT TAT GCT GTG GCC AAA CTA GGA CCA GGA GAG AAA TAT GAG CTG CAC GCT GGA ACT
601     631
GAG TCT ACC CCG AGC GTC GTG GTG CAT GTG TGT GAC AGC GAC ATG GAG AGG GAG GAG GAC
661     691
CCA AAG ACT TCC CCA AAG CCA AAA ATC AAT CAG ACC CGG CGG CCT GGC CTG CCA CCC TTC
721
GGT CAC TGA

```

coding sequence: 729 nucleotides

## murine Csp1 (Seq ID NO: 4)

1/1  
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 M E E V D L Q D L P S A T I A C H L D P  
 61/21  
 CGC GTG TTC GTG GAC GGC CTG TGC CGG GCC AAA TTT GAA TCC CTC TTC AGA ACA TAT GAC  
 R V F V D G L C R A K F E S L F R T Y D  
 121/41  
 AAG GAC ACC ACC TTC CAG TAT TTT AAG AGC TTC AAA CGT GTC CGG ATA AAC TTC AGC AAC  
 K D T T F Q Y F K S F K R V R I N F S N  
 181/61  
 CCC TTA TCT GCA GCC GAT GCC AGG CTG CGG CTG CAC AAG ACC GAG TTC CTG GGG AAG GAA  
 P L S A A D A R L R L H K T E F L G K E  
 241/81  
 ATG AAG TTG TAT TTT GCT CAG ACT TTA CAC ATA GGA AGT TCA CAC CTG GCT CCG CCC AAT  
 M K L Y F A Q T L H I G S S H L A P P N  
 301/101  
 CCC GAC AAA CAG TTC CTC ATC TCC CCT CCG GCC TCT CCT CCC GTT GGC TGG AAA CAA GTA  
 P D K Q F L I S P P A S P P V G W K Q V  
 361/121  
 GAA GAT GCC ACC CCC GTC ATA AAT TAC GAT CTT TTA TAT GCC ATC TCC AAG CTG GGG CCA  
 E D A T P V I N Y D L L Y A I S K L G P  
 421/141  
 GGA GAG AAG TAT GAA CTG CAT GCA GCG ACA GAC ACC ACT CCC AGT GTG GTG GTC CAC GTG  
 G E K Y E L H A A T D T T P S V V V H V  
 481/161  
 TGT GAG AGT GAC CAA GAG AAT GAG GAG GAA GAG GAA GAG ATG GAG AGA ATG AAG AGA CCC  
 C E S D Q E N E E E E E E M E R M K R P  
 541/181  
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 K P K I I Q T E R P E Y T P I H L S \*

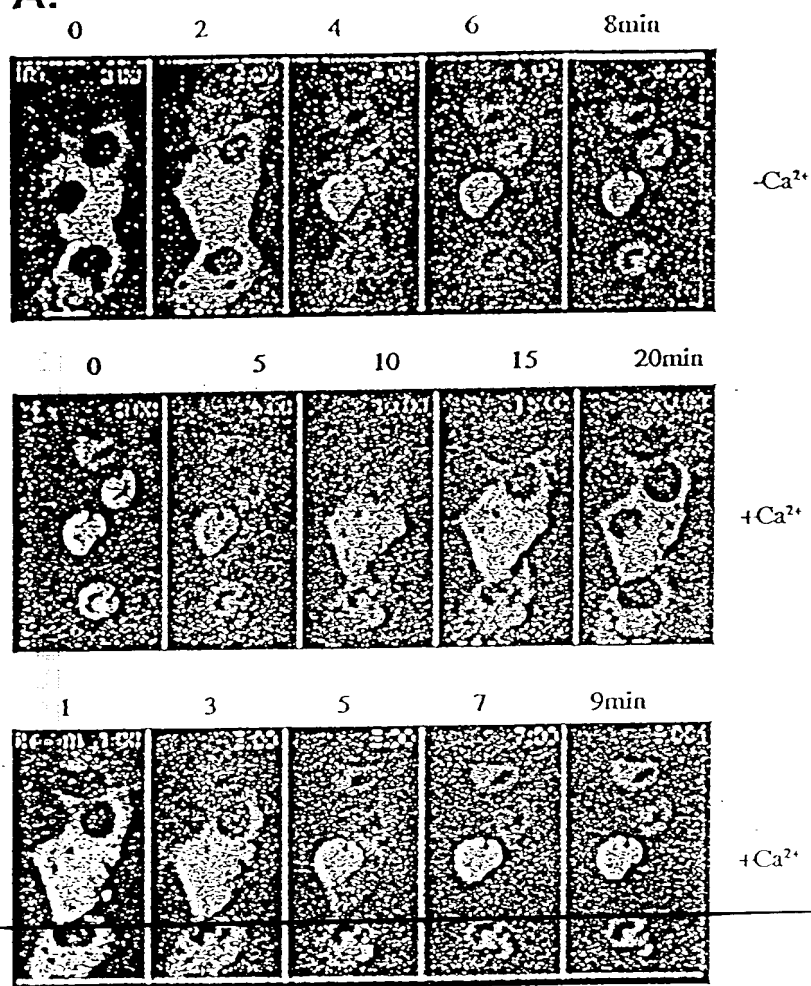
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murine Csp2 (S. ID NO: 5)

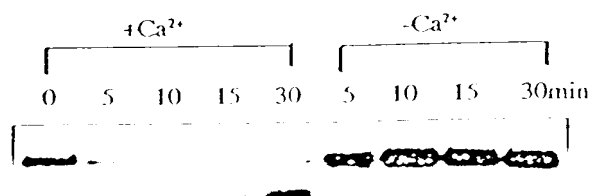
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 E F V D P R V R P R V P L G Q Q A S I P  
 61/21  
 GAA GAT GGG GGA CTT TTC TTC CTC TGC TGC ATA GAC AGA GAC TGG GCT GTC ACT CAG TGT  
 E D G G L F F L C C I D R D W A V T Q C  
 121/41  
 TTT GCT GAA GAG GCC TTC CAA GCA CTC ACT GAC TTC AGT GAT CTC CCC AAC TCA TTG TTT  
 F A E E A F Q A L T D F S D L P N S L F  
 181/61  
 GCC TGC AAT GTT CAC CAG TCT GTG TTT GAA GAA GAG GAG AGC AAG GAA AAA TTC GAG GGA  
 A C N V H Q S V F E E E E S K E K F E G  
 241/81  
 CTG TTC CGG ACC TAT GAT GAA TGT GTG ACG TTC CAG CTG TTT AAG AGT TTC CGA CGG GTT  
 L F R T Y D E C V T F Q L F K S F R R V  
 301/101  
 CGA ATA AAT TTC AGC CAT CCC AAA TCT GCA GCC CGT GCC CGG ATA GAG CTT CAT GAG ACT  
 R I N F S H P K S A A R A R I E L H E T  
 361/121  
 CAG TTC AGA GGG AAG AAG CTA AAA CTC TAC TTC GCC CAG GTC CAG ACC CCA GAG ACA GAT  
 Q F R G K K L K L Y F A Q V Q T P E T D  
 421/141  
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 481/161  
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 S S P S V G W K P I S D A T P V L N Y D  
 541/181  
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 L L Y A V A K L G P G E K Y E L H A G T  
 601/201  
 GAG TCT ACC CCG AGC GTC GTG GTG CAT GTG TGT GAC AGC GAC ATG GAG AGG GAG GAG GAC  
 E S T P S V V V H V C D S D M E E E E D  
 661/221  
 CCA AAG ACT TCC CCA AAG CCA AAA ATC AAT CAG ACC CGG CGG CTT GGC CTG CCA GCA TTT  
 P K T S P K P E I N Q T R R P G L P P F  
 721/241  
 GGT CAC TGA  
 G H \*

242 amino acids and 729 nucleotides

A.



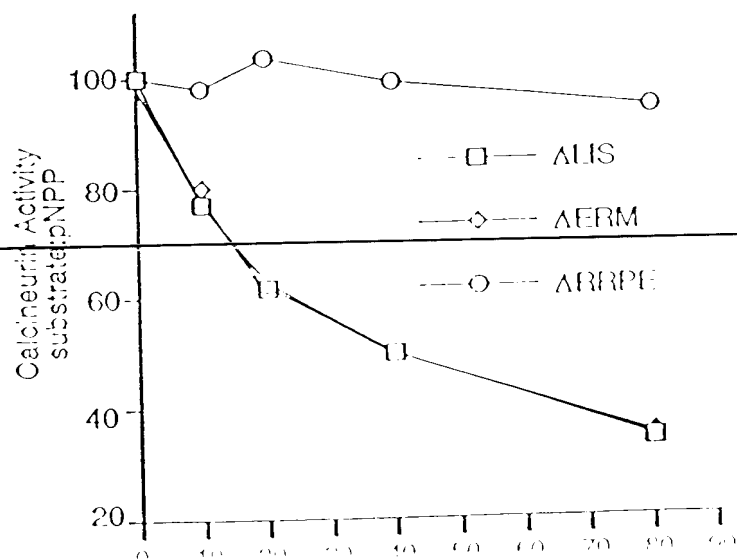
B.



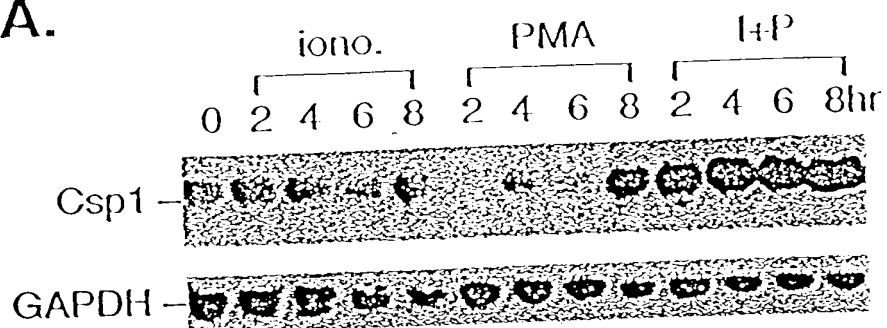


DARPP32 RQVEMT<sup>\*\*\*</sup>RRRRPTPA  
 Inhibitor-1 EA<sup>\*\*\*</sup>AEQ<sup>\*\*\*</sup>IRRRPTPA  
 Phosphorylase kinase SEIKQVEFRR<sup>\*\*\*</sup>LSIS  
 RII VPIPGRFDRRV<sup>\*\*\*</sup>SVC  
 CnA-AI RINERMPPRR<sup>\*\*\*</sup>DAMP  
 Csp1 PKPKIIQT<sup>\*\*\*</sup>RRPEYT  
 Csp2 PKPKINQT<sup>\*\*\*</sup>RRPGLP

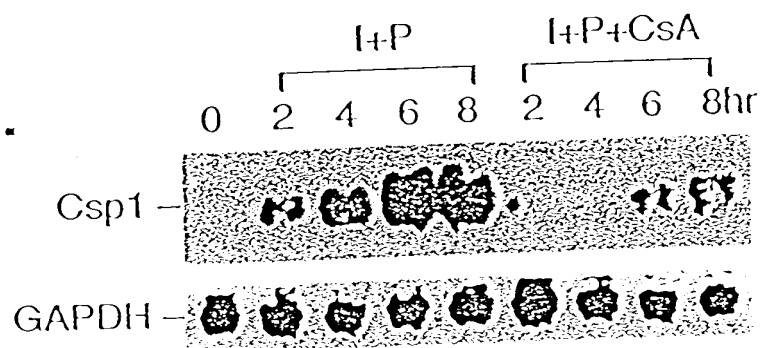
B.



**A.**



**B.**





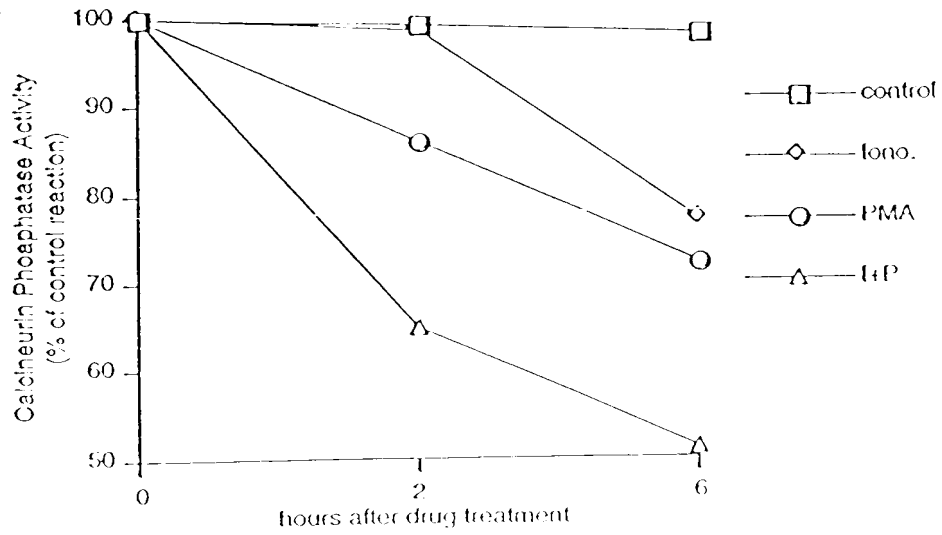


Figure 18

Murine Csp3 (SEQ ID No: 22)  
cDNA Nucleic acid sequence (coding)

atgetccgagacagcctgaaatcttggaaatgacagccagtcagacctctgtagcagcgaccaggaggagggaagaggagatggcttcggg  
gaaaatgaggacggactggaagagatgatggacctaaagtacctgcccacctcactctttgcttgcaagtgtccatgaagcagtggttgaggt  
ccaagagcaaaaggagagggttgaggccctgttcacctctacgatgaccagggtcacattccagttgttcaagagtttcgcagagtgaggat  
caacttcagcaagcccgcagagcgcggatagagtcacagagtgagttccacggacggacggaagctgaagctttacttcgcacaggtgca  
gggtgccggggaggccccgggacaagtctacttactgccaccacaaccaccaagcagttcctcactccccctcccgttcacccccgtgg  
gggtggaagcagagtgaagatgcgatgccagtgatcaactatgacctgctctgcgctgtctccaagctgggcccaggggagaaatacgaac  
tgcacgcgggaaccgagtcacccccagtggtggtggcgcgctgtgagagcgaactgaagagggaagaagacacaaaaatccaaaa  
cagaaaatcacgcagacgcggcgccccggagggtccacggcggcactgagtgagcggctggactgtgcactctga

Figure 19

cDNA nucleic acid sequence  
(entire coding + 5' and 3' UTR) (SEQ ID No: 23)

gccgctgcggccccgcgttgagggcgtggtggctccgggtggtgagggctgtccgccccaggccgcgctcgtggg  
catccccctcgggcctctccccctcgagcgcacagaagtatctggcaggcatcctagaactttacagagaagatgctc  
cgagacagcctgaaatcttggaatgacagccagtcagacctctgtagcagcgaccaggaggaggaagaggagatg  
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tccatgaagcagtgtttgaggtccaagagcaaaaggagagggtttgagggccctgttcacctctacgatgaccaggtea  
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agagtgagttccacggacggaagctgaagctttacttcgcacaggtgcaggtgtccggggaggccccgggacaagtc  
ctacttactgccaccacaaccaccaagcagttcctcatctccccctcccgcttcacccccgtgggggtggaagcagagt  
gaagatgcgatgccagtgatcaactatgacctgctctgcgctgtctccaagctgggcccagggggagaaatacgaact  
gcaegcgggaaccgagtcacccccagtggtggtgcacgtctgtgagagcgaaactgaagaggaagaagacac  
aaaaaatccaaaacagaaaatcacgcagacgcggcgccccggaggctcccacggcggcactgagtgagcggctgg  
actgtgcactctgagcggctgcggtgcctgccgcgcctgcctgtcccaccactacagctgcgcctgtctaggagcaca  
gcccagggatgctcttgcacccgtcag

Figure 20

Murine Csp3 (SEQ ID NO: 24)  
Amino acid sequence

MLRDSLKSWNDSQSDLCSSDQEEEEEMVFGENEDGLEEMMDLSDLPTSLFACSVHEAV  
FEVQEQKERFEALFTLYDDQVTFQLFKSFRRVRINFSKPARARIELHESEFHGRKLKLYF  
AQVQVSGEARDKSYLLPPQPTKQFLISPPASSPVGWKQSEDAMPVINYDLLCAVSKLGP  
GEKYELHAGTESTPSVVVHVCESETEEEEDTKNPKQKITQTRRPEAPTAALSERLDCALZ

Figure 21 Identification of a Third Calcipressin Family Member, Csp3

```

csp2      1  -----HDCDYSTLVACTVDVETET
csp3      1  HLRDSLKSWNDSDCLCSSDQEEEEEMVFGENEDGLEEMMDLSDLPTSLFACSTHEATFE
csp1      1  -----MEEVDLQDLPSATTACHLDPRVFV

csp2     20  HQEVKEKFEGLFRTYDECVTTFQLFKSFRRYRINFSEPKSAARARIELHETQFRGKKLKY
csp3     61  YQEQKERFEALFTLYDDQVTTFQLFKSFRRYRINFSEPK---ARARIELHESEPHGRKKLKY
csp1     25  DGLCRAKFESLFRTYDKDTTFQTFKSFERIRINFSEPLSAADARLRLHKTFLGKHKLY

csp2     80  FAQVQTPETDGDGLHLAPPQPAKQFLISPPSSPSVGVKPISDATPVLNYDLLYAVAKLGP
csp3    118  FAQVQTSGEARDKSYLLPPQPTKQFLISPPASSPVGVKQSEDAHPYINYDLLCAVSKLGP
csp1     85  FAQTLHIGS----SHLAPPDPDKQFLISPPASSPVGVKQVEDATPVIINYDLLYATSKLGP

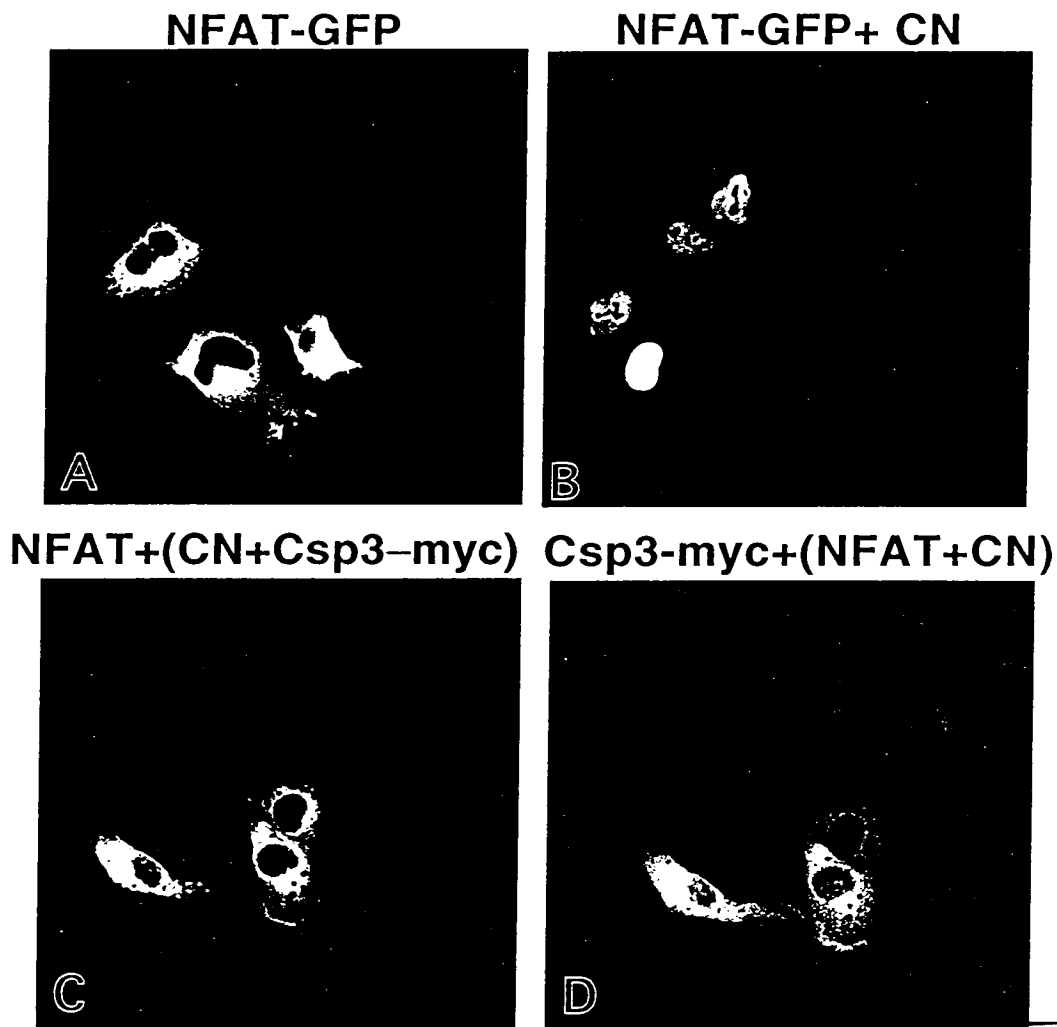
csp2    140  GEKYELHAGTESTPSVYVHYCDSDMEREDPETS-----PKPKILQTRRPGLPPFVSH--
csp3    178  GEKYELHAGTESTPSVYVHYCESETEEEEDTKI-----PKQKITQTRRPEAPTAAALSER
csp1    141  GEKYELHAATDTTPSVYVHYCESDQELEEEEEMERMKRPKPKIIQTRRPEYTPPIHLS--

csp2      -----
csp3    232  LDCAL
csp1      -----

```

A third calcipressin family member, termed csp3, was cloned from murine T cells and found to have high sequence homology with csp1 and csp2.

**Figure 22    Calcipressin 3 Inhibits Calcineurin Mediated Translocation of NFAT**

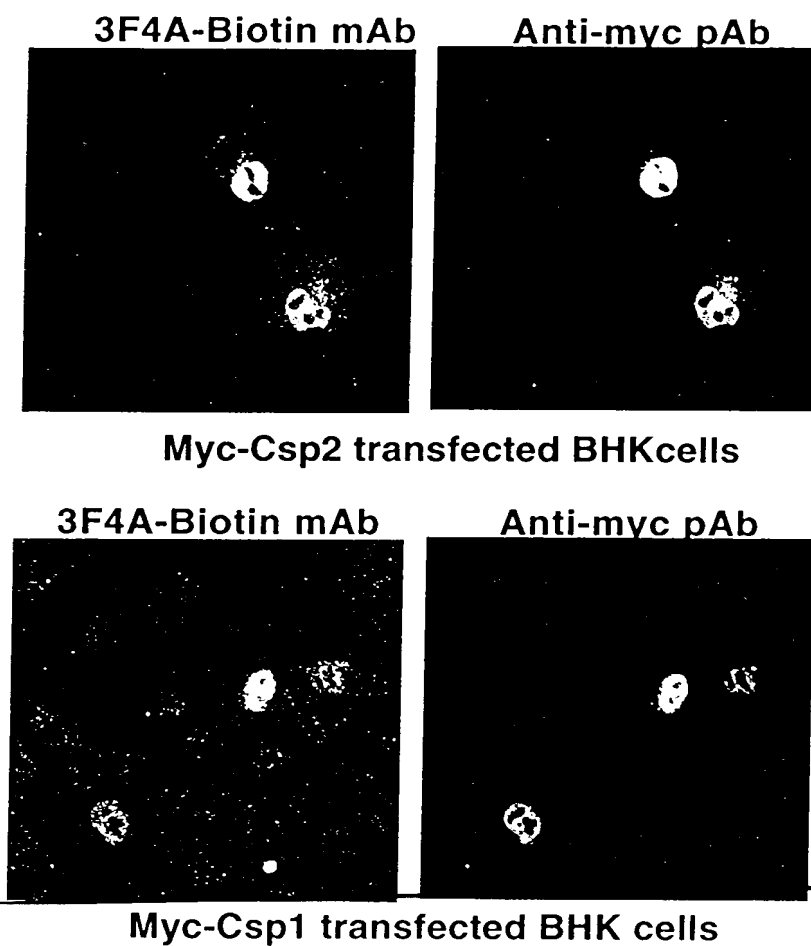


Panel A demonstrates the cytoplasmic expression pattern of the transcription factor NFAT tagged with green fluorescent protein (GFP) in the absence of stimulus. Upon co-expression of calcineurin (CN), NFAT shuttles into the nucleus as seen in panel B.

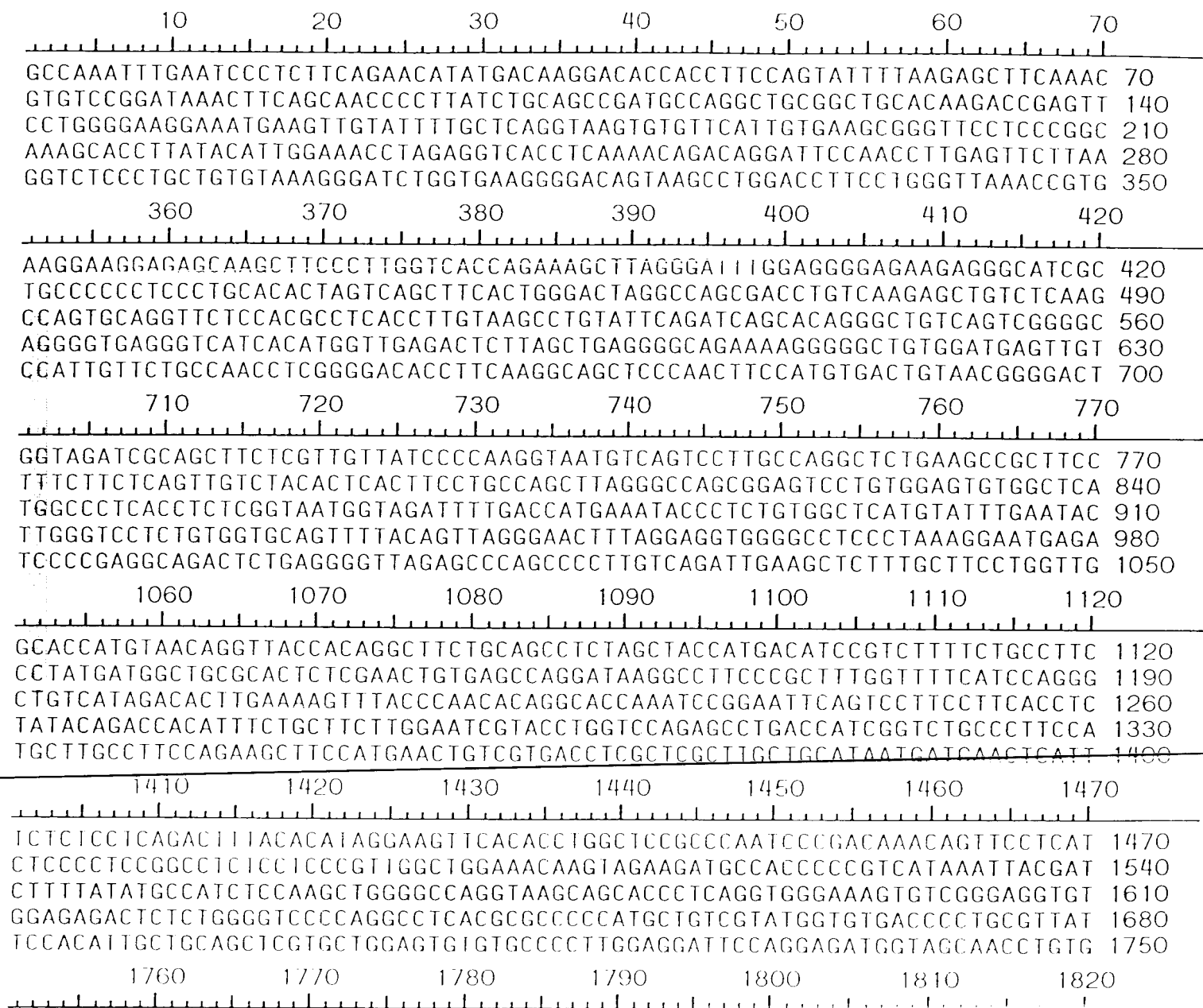
Panel C demonstrates the cytoplasmic expression of NFAT in the presence of calcineurin and calcipressin 3 (Csp3), suggesting inhibition of CN activity.

Panel D demonstrates the nuclear translocation of NFAT in the presence of CN and Csp3-myc, as indicated in panel C. Immunostaining with anti-myc antibody is used to detect the myc-tagged Csp3 protein.

**Figure 23 . Generation of anti-Csp2 and anti-Csp1  
Monoclonal Antibodies**



Monoclonal antibodies (mAb) were generated against Csp1 and Csp2. 3F4A mAb was biotinylated and demonstrated to recognize cells transfected with both myc-tagged csp2 (top panel) and csp1 (bottom panel), as verified by immunostaining with a myc pAb.



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Figure 24



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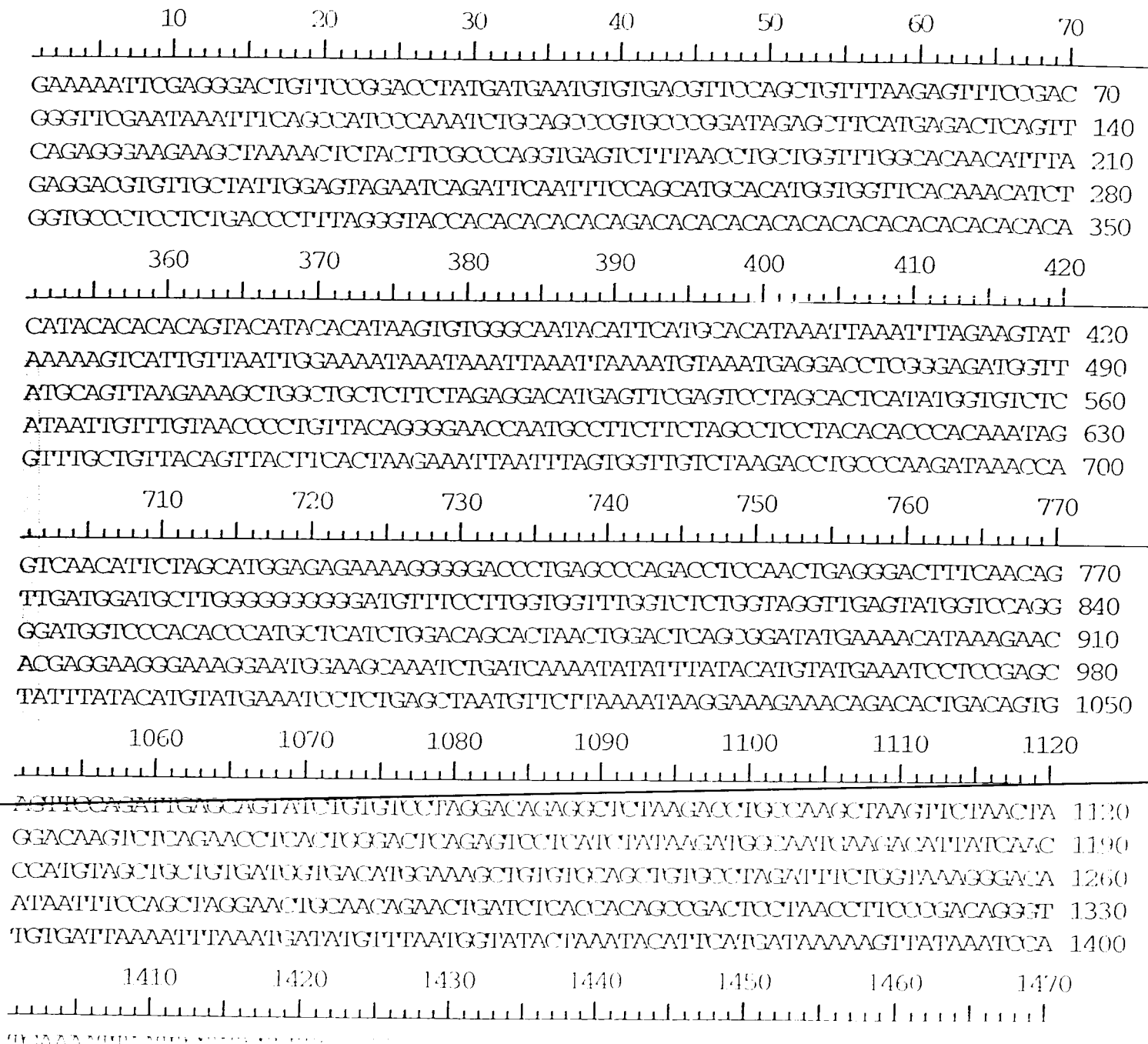


Figure 25

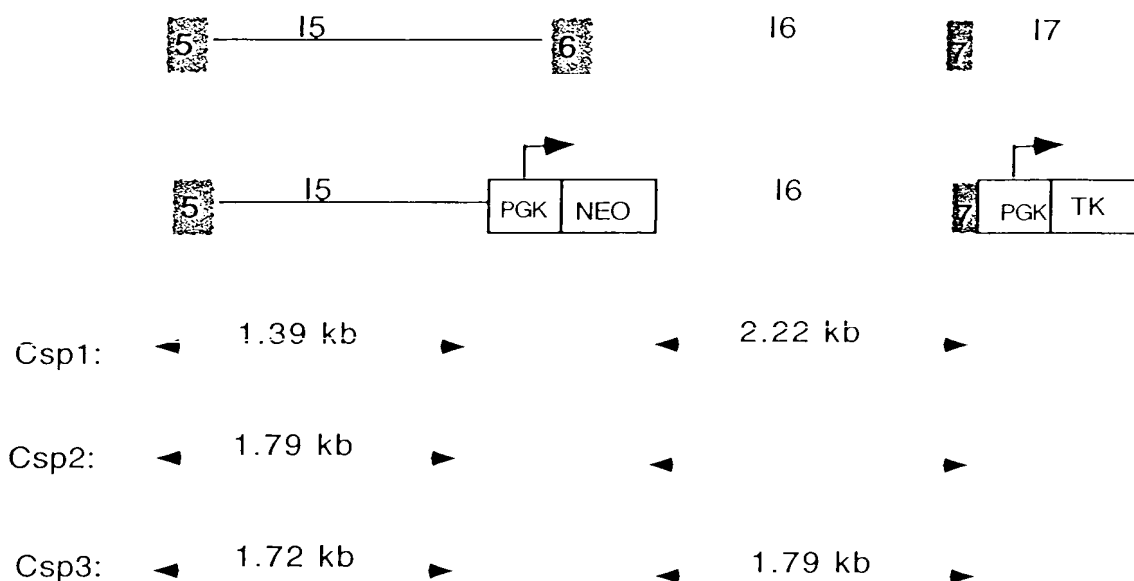
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1. *Is there a need for a new approach to the study of the history of the United States?*

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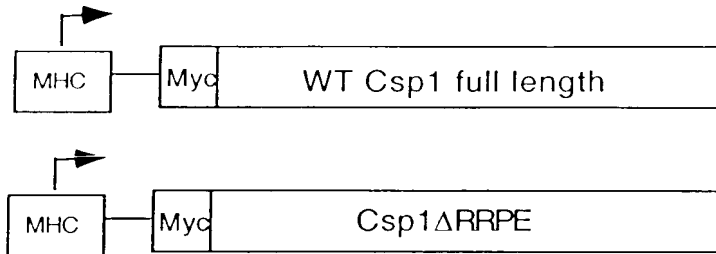
Figure 27 . Schematic Representation of the Gene-targeting Vectors Used to Disrupt the Csp1, -2, and -3 Genes



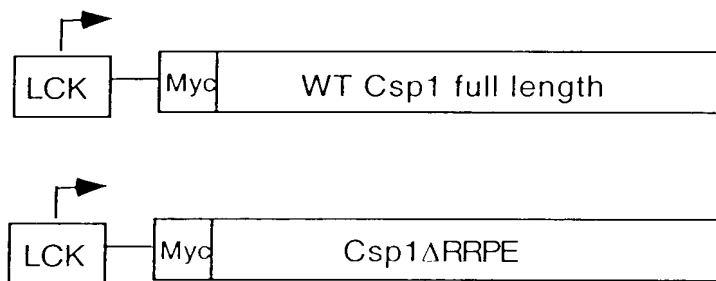
This schematic diagram shows the organization of the Csp genes (top) and the targeting vectors (middle) constructed to disrupt the Csp genes. Our targeting vector will replace exon 6 with the neomycin drug resistance genes. This exon contains the start of the inhibitory, or c-terminal domain of all three genes which should effectively destroy the calcineurin inhibition activity. The genomic structure of all three genes is relatively similar with different size introns (I5, I6). Exons are denoted by the shaded boxes with numbers.

Figure 28 **Constructs Used to Generate Tissue-Specific Expression of Csp1 in Transgenic Mice**

**Cardiac Specific Expression:**



**T-Cell Specific Expression:**



This schematic diagram demonstrates the constructs injected into blastocysts to generate transgenic mice. Wild-type full length myc-tagged Csp1 under the control of a myosin heavy chain (MHC) promoter (top half) will ensure cardiac specific expression. Similarly Csp1 with the sequence element, amino acids, 188-191, "RRPE" deleted is also expressed under the MHC promoter.